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WITNESS my hand this Eleventh day of May 1999

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Howard Milne Chandler

**AUSTRALIA**
**Patents Act 1990**

# PROVISIONAL SPECIFICATION
for the invention entitled:

"Sample Collection Method"

The invention is described in the following statement:

# SAMPLE COLLECTION METHOD

## FIELD OF THE INVENTION

This invention relates to a method for collecting a sample, for subsequent use in the detection of an analyte in the sample. In one particular embodiment, this invention relates to a method for collecting a faecal sample for the purposes of subsequent occult blood detection in the sample.

The present invention also extends to an assay kit which is particularly suitable for the purposes of detection of occult blood in a faecal sample.

## BACKGROUND OF THE INVENTION

A well known and widely-used clinical reagent for the detection of occult blood in a sample, particularly a faecal sample, is guaiac (also known as gum guaiac or resin guaiac). When used in association with an appropriate developer solution, guaiac provides a colorimetric assay system for detecting haemoglobin in the sample. Such tests are commercially available, for example, Hemoccult II and Hemoccult II Sensa (SmithKline Diagnostics, San Jose, California, USA).

Prior Australian Patent No. 665956 (International Patent Application No. PCT/US92/04425) notes that among the many analytical systems used for detection and/or determination of analytes, particularly analytes of biological interest, are chromatographic assay systems. Among the analytes of biological interest frequently assayed with such systems are:

1. hormones, such as human chorionic gonadotropin (hCG), frequently assayed as a marker of human pregnancy;
2. antigens, particularly antigens specific to bacterial, viral, and protozoan pathogens, such as *Streptococcus*, hepatitis virus, and *Giardia*;
3. antibodies, particularly antibodies induced as a result of infection with

pathogens, such as antibody to the bacterium *Helicobacter pylori* and to human immunodeficiency virus (HIV);

4. other proteins, such as haemoglobin, frequently assayed in determinations of faecal occult blood, an early indicator of gastrointestinal disorders such as colon cancer;

5. enzymes, such as aspartate aminotransferase, lactate dehydrogenase, alkaline phosphatase, and glutamate dehydrogenase, frequently assayed as indicators of physiological function and tissue damage;

6. drugs, both therapeutic drugs, such as antibiotics, tranquillisers and anticonvulsants, and illegal drugs of abuse, such as cocaine, heroin, and marijuana; and

7. vitamins.

Such chromatographic systems are frequently used by physicians and medical technicians for rapid in-office diagnosis and therapeutic monitoring of a variety of conditions and disorders. They are also increasingly used by patients themselves for at-home monitoring of such conditions and disorders.

Among the most important of such chromatographic systems are the "thin layer" systems in which a solvent moves as a solvent front across a thin, flat absorbent medium. Among the most important of tests that can be performed with such thin layer systems are immunoassays, which depend on the specific interaction between an antigen or hapten and a corresponding antibody. The use of immunoassays as a means of testing for the presence and/or amount of clinically important molecules has been known for some time.

Chromatographic techniques used in conjunction with immunoassays include a procedure known as immunochromatography. In general, this technique uses a disclosing reagent or particle that has been linked to an antibody to the analyte to be assayed, forming a conjugate. This conjugate is then mixed with a specimen and, if

the analyte to be assayed is present in the specimen, the disclosing reagent-linked antibodies bind to the analyte to be assayed, thereby giving an indication that the analyte to be assayed is present. The disclosing reagent or particle can be identifiable by colour, magnetic properties, radioactivity, specific reactivity with another molecule, or another physical or chemical property. The specific reactions that are employed vary with the nature of the analyte being assayed and the sample to be tested.

The present invention is particularly, but not exclusively, directed to collection of faecal samples for occult blood detection, for example in screening for colorectal cancer. As previously described, guaiac testing provides a colorimetric assay system for detection of haemoglobin in a sample, however because of the large number of false positives obtained in guaiac testing, in screening programs the use of two or three guaiac tests has been recommended, confirmed when positive by an immunological test for human haemoglobin (Favennic L., Kapel N., Meillet D., Chochillon C. and Gobert J.G., *Annales de Biologie Clinique*, **50**(5):311-3, 1992). More recently, a combination of guaiac and immunological testing has been suggested (Allison, J.E., Tekawa, I.S., Ransom, L.J. and Adrian, L.L. *N. Engl. J. Med.*, **334**:155-9, 1996).

It is an object of the present invention to provide a sample collection method which is simple and economic, and which enables subsequent detection and/or determination of analyte in the sample to be readily carried out, for example using a guaiac test, and/or an immunochromatographic or other immunodiagnostic procedure.

## SUMMARY OF THE INVENTION

In accordance with the present invention, there is provided a method for collecting a sample of faecal material, comprising contacting the surface of the faecal material or fluid surrounding the faecal material with a brush or brush-like device, wherein the sample is collected within the bristles of the brush or brush-like device.

The term "brush" as used herein is used to denote device comprising a handle member, usually elongate, and a clump, bunch or group of bristles, hair or other similar flexible or semi-flexible elongate strands, laminar flaps or the like attached to the handle member. The term "brush-like device" is used herein to denote a device which is similar to a brush in that it includes a bunch, clump or group of bristles, hair or other similar flexible or semi-flexible elongate strands, laminar flaps or the like. Whilst reference is made throughout the present specification to the collection of a sample within the bristles of a brush or brush-like device, it is to be understood that the reference to "bristles" is used to include the hairs or other similar flexible or semi-flexible elongate strands, laminar flaps or the like of a brush or brush-like device.

Preferably, the bristles of the brush or brush-like device will have a length of about 0.5 to 3 cm long, more preferably a length of 1 to 2 cm.

In another embodiment, the present invention also extends to an assay kit which comprises a sample collection device which is a brush or brush-like device, together with means for detection of an analyte in a sample.

Such an assay kit is particularly suited for use in detection of occult blood in a faecal sample. The detection of occult gastrointestinal bleeding is a common method for screening for colorectal cancer. Commonly referred to as the faecal occult blood (FOB) test, a variety of formats are known in the art (see, for example, US Patent Nos. 3996006; 4225557; 4789629; 5064766; 5100619; 5106582; 5171528; 5171529; and 5182191). The majority of test formats are based on the chemical detection of the heme groups present in faecal material as a breakdown product of blood. In such tests, the pseudoperoxidase nature of the heme group is used to catalyse a colorimetric reaction between an indicator dye and peroxide. The oxygen sensitive dye can be gum guaiac, orthodianisidine, tetramethylbenzidine, or the like, with guaiac being preferred.

The means for detection of an analyte in a sample which is incorporated into an assay kit as described above may, for example, be means for carrying out a guaiac test for the detection of occult blood in the sample. Alternatively, or additionally, the means for detection of an analyte in a sample may be means for detection of occult blood (or other diagnostic antigens) in the sample by means of a chromatographic procedure, particularly by an immunochromatographic or other immunodiagnostic procedure which is well known in the art. Suitable immunochromatographic procedures are described, by way of example, in US Patent Nos. 5591645 and 5622871, the disclosures of which are incorporated herein by reference.

Throughout this specification, unless the context requires otherwise, the word "comprise", and or variations such as "comprises" or "comprising", will be understood to imply the inclusion of a stated integer or step or group of integers or steps but not the exclusion of any other integer or step or group of integers or steps.

DETAILED DESCRIPTION OF THE INVENTION

In the most preferred embodiment, the present invention relates to the use of a brush as a sample device for faecal and particularly stool, samples in water, particularly for the detection of occult blood (or other diagnostic antigens) as an indicator of colorectal cancer (CRC) or its precursor conditions.

Most existing faecal occult blood tests (FOBTs) use a sampling stick or paddle to take smears directly from the surface of a collected faecal sample. Many CRCs or their precursors (e.g. adenomas > 1cm), bleed into the lumen of the small intestine. As these malignancies arise as protrusions from the wall of the intestine they make contact with the surface of the stool in their region of contact as the stool passes that point. The blood, therefore, may not be evenly distributed through or over the stool. As a result, existing tests that rely on surface sampling of the stool may or may not sample from that portion of the stool where blood is present.

If the stool is sampled by a suitable sampling device once it is in the water of the toilet bowl, there is a better opportunity to gain a representative sampling of the whole stool. This is particularly the case where a small brush (e.g. a small artist's paint brush having bristles about 1 cm in length) is used for sampling. A brush may be used to "paint" the surface of the stool so as to displace any blood on the surface of the stool into the water surrounding the stool. The bristles of the brush will be relatively "open" during this brushing and sampling process, but will "close" as the brush is withdrawn from the water, thereby keeping within the interstitial spaces of the bristles a sample of the water, (and any blood), surrounding the stool. This sample may then be transferred to a suitable assay device for subsequent testing.

By way of contrast, if another sampling device, such as a swab, was used for sampling, water would infiltrate the fibre windings of the swab on its first contact with the bowl water. In this case there would be little chance of effective displacement of the infiltrated water by any blood-containing water in the vicinity of the stool, and as a result the sampling procedure would not effectively sample any such blood-containing water.

Alternatively, if a solid sampling device such as a loop or barbed probe was used, the water sampled from around the stool would be lost as the device was withdrawn through the water of the bowl, and once again the sampling procedure would not effectively sample any blood-containing water.

Further features of the present invention are more fully described in the following Example(s). It is to be understood, however, that this detailed description is included solely for the purposes of exemplifying the present invention, and should not be understood in any way as a restriction on the broad description of the invention as set out above.

## EXAMPLE

The suitability of the brush for sampling blood in water has been shown to be effective by several means:

1. Blood (10 µL) was added to water 50 mL) in a beaker. After the blood settled to a discrete drop at the bottom of the beaker, a brush (#5, LiFung) was first used to sample the surface water from the beaker. This sample tested negative in a faecal occult blood (FOB) test (Enterix). A second brush was shown to be capable of selectively sampling sufficient of the blood to be detected in a similar FOB test.

2. A stool sample was injected with blood (50 µL) so that the blood was sequestered within a crevice in the stool. The stool was added to the toilet bowl and brushes as described above were used to sample:

   (a) The water of the bowl.

   (b) The water surrounding the stood after the surface of the stool was "brushed".

   When tested in FOB tests, samples (a) tested negative for blood, whereas samples (b) tested positive. In this experiment it may be expected that the sequestered blood would have been missed by conventional sampling of the stool surface with a stick or paddle.

3. The Table below shows the results of a series of experiments to test the effectiveness of brush sampling of stool samples. Blood was added directly to normal stool samples, before or after the deposition of the stools into the toilet bowl. Normal stools and the bowl water before stool addition were also

sampled. In each case samples collected by the brush method were tested for the presence of blood by an FOB test (Enterix).

TABLE

| FOB Test Results | Bowl Water | Normal stool (i.e. no addition) | 25 µL blood added | 50 µL blood added | 100 µL blood added |
|---|---|---|---|---|---|
| No. positive | | | 4 | 15 | 27 |
| No. negative | 2 | 15 | | | |

As shown in the Table, all toilet bowl water and normal stool samples tested negative in the FOB test, whereas all samples with added blood (≥ 25 µL) gave a positive test result. These results compare favourably with the sensitivity and specificity data reported by Rosen (1997) with tests that use direct stool sampling with a sampling stick.

Persons skilled in this art will appreciate that variations and modifications may be made to the invention as broadly described herein, other than those specifically described without departing from the spirit and scope of the invention. It is to be understood that this invention extends to include all such variations and modifications.

Dated this 28th day of April 1998

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